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# PHYSICO-CHEMICAL CHARACTERISATION OF BREWERY EFFLUENT AND ITS DEGRADATION USING NATIVE FUNGUS *ASPERGILLUS NIGER*

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## ABSTRACT

**Physicochemical parameters (pH, EC, TSS, TDS, BOD, COD, Total Hardness (TH), Chloride, Copper and Zinc) of treated brewery effluent were estimated. The results of the study revealed that parameters were found to be above the permissible limits for disposal prescribed by CPCB. Bacteria and Fungi present in the effluent were isolated and identified. Laboratory scale degradation of 100% treated brewery effluent was carried out using native fungus - *Aspergillus niger* as it is efficient in degrading the effluent in such a way that the effluent meet the permissible limits so that it can be reused for agricultural and aquacultural purposes.**

## INTRODUCTION

Water resource is most often affected by various industrial effluents which are generally discharged into water courses either treated or untreated. Most of the industries like distilleries, tanneries, paper and pulp, milk and breweries produce their waste in the form of effluent (Nagarajan and Shasikumar 2002). Industrial effluents are important source of organic pollution. The disposal of the industrial wastes directly into aquatic bodies burdens the ecosystem (Aftab and Noorjahan, 2006). The chemicals and heavy metals present in these effluents produce harmful effects on animals and plants (Nagarajan and Shasikumar, 2002). Brewery industry is one of the important industries that cause water pollution. It is one of the largest industrial users of water. The brewery industry consumes much water about 10 gallons of processed water/gallon of product. Eventhough substantial technology improvements have been made in the past it has been documented that approximately 3-10 litres of waste effluent is generated/litre beer produced in breweries. Discharge of such brewery effluent with toxic substances without any prior treatment into land or in to any water bodies will pose ground water contamination and aquatic pollution. Hence it becomes necessary to treat the effluent before its disposal. Biological treatment is the most widely used method for removal as well as partial or complete stabilization of biologically degradable substances present in waste waters (Grueninger *et al*. 1984). *Aspergillus niger* have potential use as biosorbent for removal of heavy metals particularly chromium from industrial waste waters which is in accordance with the reports of Akthar and Mohan (1995) and Sujatha *et al*. (1995). It is also supported by the work of Aftab and Noorjahan (2006).

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Taking into consideration of all the above said investigations carried out by many researchers research pertaining to degradation of brewery effluent using microbes is wanting. Hence an investigation has been carried out to analyse the physico-chemical parameters of industry treated brewery effluent, to isolate and identify the microbes (bacteria and fungi) present in the industry treated brewery effluent and to ascertain the treatment using native fungus, *Aspergillus niger.*

## MATERIALS AND METHODS

Industry treated brewery effluent was collected in 2½ litres polythene containers from discharge point wherein effluent from all the stages of processing are released together and brought to the laboratory with due care and stored in refrigerator for further analysis.

Physico chemical parameters such as Colour, Odour, pH, Electrical Conductivity (EC), Total Suspended Solids (TSS), Total Dissolved Solids (TDS), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Hardness (TH), Chloride (Cl), Copper (Cu) and Zinc (Zn) of industry treated brewery effluent were analysed by following the Standard methods of APHA (1989 and 1998). Industry treated brewery effluent of about 1 litre was collected in sterile bottles and brought to the laboratory. Microbial analysis were carried out on the same day. 1 ml of industry treated brewery effluent was diluted to 10-1 using sterile distilled water and was cultured following pour plate method using Nutrient Agar medium (NA). Bacterial species developed on the medium were observed periodically and stained using gram staining method and identified using Microbiology Laboratory Manual of Sundararaj (1997).

Bacteria present in the industry treated brewery effluent were further identified by carrying out biochemical reactions like IMVIC tests: Indole, Methyl red, Voges Proskauer and Citrate utilization test, Starch hydrolysis and Catalase test by following the procedures of Sundararaj (1997).

1 mL of industry treated brewery effluent was diluted to 10-1 using sterile distilled water and was cultured on malt extract agar (MEA) medium following pour plate method. Fungal species developed on the medium were observed periodically. The fungal colonies grown on MEA were subcultured on Potato Dextrose Agar (PDA) slants and they were stained with lactophenol cotton blue and identified using the Manual of Onions *et al*. (1981).

10 litres of brewery effluent was collected from the site for degradation process. Native fungus Aspergillus niger were selected for the degradation study. Biodegradation process was carried out by following the procedure of Noorjahan (2002). The data obtained from the experiments were analysed and expressed as mean and standard deviation. The percentage change was also calculated.

## RESULTS AND DISCUSSION

Results of the analysis of physico-chemical parameters of industry treated brewery effluent is depicted in Table 1. Results of the analysis of physico-chemical parameters showed that the brewery effluent was brownish black in colour with offensive odour. This colour and odour could be due to decomposition of organic or inorganic matter (Singh *et al*. 1998).

pH of the industry treated brewery effluent was found to be acidic. Discharge of such effluent with acidic pH into ponds, rivers, etc. for irrigation may be detrimental to aquatic biota such as zooplankton and fishes. According to Singh *et al*. (1998), highly acidic water if consumed would affect the mucous membrane and may cause metabolic alkalosis.

Industry treated brewery effluent has higher level of Electrical conductivity which could reflect the presence of organic and inorganic substances and salts that would have increased the conductivity (Robinson and Stokes, 1959; Marwaha *et al*. 1998 and Aftab and Noorjahan, 2006).

Suspended solids is found to be higher in industry treated brewery effluent (1020 mg/L) when compared to the permissible limit (100 mg/L) prescribed by CPCB (1995) for effluent discharge. High amount of TSS elevated the density and turbidity of water thereby affects osmoregulation and interferes with photosynthesis by preventing sunlight (Kalita *et al*. 2003).

High levels of TDS (2100 mg/L) is recorded in this study may be due to high inorganic salt contents present in the industry treated effluent and also renders it unsuitable for irrigation hence further treatment or dilution would be required (Goel, 1997 and Elizabeth *et al*. 2006).

The present study reveal high levels of BOD in the industry treated brewery effluent which may be due to the presence of considerable amount of organic matter. High BOD levels have also been reported in tannery effluent by Kulkarni (1992) and in fertilizer effluent by Aftab and Noorjahan (2006).

The present study showed high levels of COD which surpassed the standards limit for COD (250 mg/L) prescribed by CPCB (1995) for effluent discharge into inland surface waters thereby indicating large quantity of organic and inorganic matter present in the effluent which is supported by the work of Kansal *et al*. (2005).

Levels of Total Hardness is found to be higher in industry treated brewery effluent (500 mg/L) when compared to the permissible limit (300 mg/L) prescribed by CPCB (1995) for effluent discharge which is mainly due to the presence of carbonates and bicarbonates of calcium and magnesium ions and it is in accordance with the findings of Rajput *et al*. (2004).

With regard to chloride level the value is found to be higher in industry treated brewery effluent (534 mg/L) when compared to the permissible limit (600mg/L) prescribed by CPCB (1995). The effluent with higher level of chloride when discharged into water body will prevent the self purification of water and may harm the fishes and other aquatic life.

The industry treated brewery effluent is found to contain heavy metal pollutants such as copper and zinc. In the present study the Cu and Zn levels which are well within the permissible limit of CPCB (1995). The presence of heavy metals in the effluent produce several effects on living organisms as reported by Chukwu (2006).

Thus the analysis of physico chemical parameters of industry treated brewery effluent confirms that the wastewater although it is treated by the treatment plant of brewery industry has acidic pH and higher concentration of EC, BOD, COD, TSS, TDS, Total Hardness etc. which surpassed the permissible limits prescribed by CPCB (1995) for discharge of industrial effluent into inland surface water as well as on land for irrigation.

Moreover it also reveals that the industry treated brewery effluent is highly polluted and it has to be treated before disposal. Hence it is imperative to adopt technologies to reduce or degrade the industry treated brewery effluent. Hence study is further extended to isolate and identify the microbes (bacteria and fungi) present in the industry treated brewery effluent.

The results of microbial analysis (Table 2a) of industry treated brewery effluent reveals the occurrence of 2 bacterial species namely *Escherichia coli* and *Klebsiella pneumoniae* which is confirmed by different biochemical test that has been isolated from industry treated brewery effluent and it is supported by the work of Kutty *et al.* (2000). Prince *et al*. (2005) reported the presence of *Pseudomonas* species in the industrial effluent. Presence of *Staphylococcus aureus* is also reported in industrial effluent by Mosso *et al*. (1994) and Afen and Noorjahan (2007).

The results of fungal analysis (Table 2b) shows the occurrence of 4 species of fungi which include *Aspergillus niger, Rhizopus stolonifer, Sordaria* sp and *Syncephalastrum racemosum* from industry treated brewery effluent, which is in accordance with the work of Wahegaonkar and Sahasrabudhe (2005). Prabakar (1999) also reported the occurrence of various fungi in sugar and distillery effluent, which contains high organic load. Noorjahan *et al*. (2003) identified 7 species of fungi in both untreated and industry treated dairy effluent. Afen and Noorjahan (2007) has reported the presence of 6 species of fungi in fertilizer effluent.

Native fungus *Aspergillus niger* was selected for biodegrading industry treated brewery effluent for 96 hrs. Results of the present investigation (Table 3) reveals that the colour and odour of industry treated brewery effluent before degradation are brownish black and has offensive odour. But after degradation of the effluent for 96 hrs, using *Aspergillus niger*, the colour changes to colourless with almost odourless condition of the effluent. This may be due to the action of fungus *Aspergillus niger* which decomposed the toxic pollutants present in the effluent and has changed the colour and odour of the effluent. This is supported by the work of Jamal Mohamed (2002) and Aftab and Noorjahan (2006).

pH of industry treated brewery effluent is acidic in nature before degradation but after degradation using *Aspergillus niger*, acidic nature of pH changes to neutral state, which may be due to accumulation of organic acids and also indicate the efficiency of the *Aspergillus niger* to degrade the effluent. This is in agreement with the reports of Noorjahan *et al*. (2004) and Aftab and Noorjahan (2006).

EC of industry treated brewery effluent before treatment is higher than the permissible limit of CPCB (1995) but after degrading the industry treated brewery effluent for 96 hrs, *Aspergillus niger* showed maximum reduction of EC (72.93%) in industry treated brewery effluent which indicates the efficiency of *Aspergillus niger* in degrading the industry treated effluent. This is supported by the work of Aftab and Noorjahan (2006).

TSS of industry treated brewery effluent before treatment was higher than the permissible limit of CPCB (1995) but after degrading the industry treated brewery effluent for 96 hrs. *Aspergillus niger* showed a maximum reduction of TSS (96.47%).

TDS of industry treated brewery effluent before treatment was beyond the permissible limit of CPCB

(1995). But after degrading the industry treated brewery effluent for 96 hrs, *Aspergillus niger* showed a maximum reduction of TDS (63.65%) of industry treated brewery effluent. This maximum reduction of TDS by *Aspergillus niger* may be due to consumption of inorganic and organic matter by microbes for their food (Hulmes *et al*. 1993).

BOD of industry treated brewery effluent before degradation was higher than the permissible limit of CPCB (1995). But after degradation for 96 hrs *Aspergillus niger* showed a maximum reduction of BOD (87.39%) of industry treated brewery effluent. This maximum reduction of BOD by *Aspergillus niger* shows the efficient degrading capability of *Aspergillus niger* by degrading contaminants as they use it for their growth and reproduction (Hossain and Das, 2001).

COD of industry treated brewery effluent before treatment was beyond the permissible limit of CPCB (1995). But after degrading the industry treated brewery effluent for 96 hrs *Aspergillus niger* showed a maximum reduction of COD (64.23%) of industry treated brewery effluent which is supported by the work of Poonkothai and Parvatham (2005).

TH of industry treated brewery effluent before degradation is beyond the permissible limit of CPCB (1995). But after degradation for 96 hrs maximum reduction of TH (60%) was recorded.

Chloride of industry treated brewery effluent before degradation is beyond the permissible limit of CPCB (1995). But after degradation for 96 hrs. a maximum reduction of chloride (71.91%) was recorded.

Copper of industry treated brewery effluent before treatment is within the permissible limit of CPCB (1995). But after degradation for 96 hrs. *Aspergillus niger* reduces copper to a very low level (74.18%) thereby indicating that *Aspergillus niger* has the ca-

**Table 1.** Physico-chemical parameters of Industry treated brewery effluent.

| S.No. | Parameters | CPCB (1995) | Industry Treated Effluent |
|---|---|---|---|
| | **Physical Parameters** | | |
| 1. | Colour | Colourless | Whitish |
| 2. | Odour | Odourless | Offensive |
| 3. | pH | 5.5-9 | 5.18 |
| | **Chemical Parameters** | | |
| 4. | Electrical Conductivity (µmhos/cm) | 400 | 3695 |
| 5. | Total Suspended Solids (TSS) mg/L | 100 | 1020 |
| 6. | Total Dissolved Solids (TDS) mg/L | 2100 | 2614 |
| 7. | Biological Oxygen Demand (BOD) mg/L | 30 | 230 |
| 8. | Chemical Oxygen Demand (COD) mg/L | 250 | 643 |
| 9. | Total Hardness mg/L | 300 | 500 |
| 10. | Chloride (Cl) mg/L | 600 | 534 |
| 11. | Copper (Cu) mg/L | 3 | 0.00587 |
| 12. | Zinc (Zn) mg/L | 1.0 | 0.07570 |

**Table 2a.** Biochemical tests of bacterial culture isolated from Industry treated brewery effluent

| S.No. | Organism | Gram Staining | Methyl Red | Voges Proskauer Test | Citrate Utilization Test | Catalase Test | Indole Test | Starch Hydrolysis Test |
|---|---|---|---|---|---|---|---|---|
| 1. | *Escherichia coli* | - | + | + | + | + | + | + |
| 2. | *Klebsiella pneumoniae* | - | +. | + | + | + | + | + |

**Table 2b.** Isolation and identification of fungi from industry treated brewery effluent

| Medium | Sl.No. | Fungal Species | Industry treated brewery effluent |
|---|---|---|---|
| Malt Extract Agar | 1. | *Aspergillus niger* | + |
| | 2. | *Rhizopus stolonifer* | + |
| | 3. | *Sordaria* sp | + |
| | 4. | *Syncephalastrum racemosum* | + |

+ = Present; - = Absent

**Table 3.** Analysis of physico-chemical parameters of industry treated brewery effluent (100%) before (control) and after degradation using *A.niger*

| SI. No. | Parameters | CPCB (1995) (Values) | | Effluent before biodegradation (Control) | Effluent after biodegradation |
|---|---|---|---|---|---|
| | Physical Parameter | | | | |
| 1. | pH | 5.5 - 9.0 | Mean ± S.D | 5.18 ±1.632 | 7.1 ±0.082 |
| | | | % Change | | 37.06% |
| | Chemical Parameter | | | | |
| 2. | Electrical Conductivity (EC) µmhos/cm | 400 | Mean ± S.D | 3695 ±4.08 | 1000 ±0.976 |
| | | | % Change | | 72.93% |
| 3. | Total Suspended Solids (TSS) mg/L | 100 | Mean ± S.D | 1020 ±16.32 | 36 ±9.41 |
| | | | % Change | | 96.47% |
| 4. | Total Dissolved Solids (TDS) mg/L | 2100 | Mean ± S.D | 2614 ±11.43 | 950 ±20.41 |
| | | | % Change | | 63.65% |
| 5. | Biological Oxygen Demand (BOD) mg/L | 30 | Mean ± S.D | 230 ±8.16 | 29 ±4.54 |
| | | | % Change | | 87.39% |
| 6. | Chemical Oxygen Demand | 250 | Mean ± S.D | 643 ±10.61 | 230 ± 12.24 |
| | | | % Change | | 64.23% |
| 7. | Total Hardness (TH) mg/L (COD) mg/L | 300 | Mean ± S.D | 500 ± 12.24 | 200 ± 10.61 |
| | | | % Change | | 60% |
| 8. | Chloride (Cl) mg/L | 600 | Mean ± S.D | 534 ±3.26 | 150 ±3.26 |
| | | | % Change | | 71.91% |
| 9. | Copper (Cu) mg/L | 3 | Mean ± S.D | 0.00581 ± 0.000021 | 0.0015 ±0.00029 |
| | | | % Change | | 74.18% |
| 10. | Zinc (Zn) mg/L | 1.0 | Mean ± S.D | 0.07566 ± 0.000037 | 0.035 ±0.0029 |
| | | | %. Change | | 53.74% |

± = standard deviation

pacity to reduce copper.

Zinc of industry treated brewery effluent before degradation is within the permissible limit of CPCB (1995). But after .degradation for 96 hrs. a very low level of Zinc (53.74%) was recorded.

Thus from the foregoing discussion it is very clear that *Aspergillus niger* play an important role in the degradation of organic and inorganic matter present in the industry treated brewery effluent.

*Aspergillus niger* degrade parameters such as EC, TSS, TDS, BOD, COD etc. From the foregoing discussion it is very clear that microbes play an important role in the biodegradation of organic and inorganic matter, their role in biodegradation explains the following criteria. Micro-organisms have enzymes that allow them to use environmental contaminants as food and hence make them ideal for degradation. Besides, their characteristics like rapid growth, metabolism and a remarkable ability to adjust to a variety of environments make them very useful in degradation. How successful are the micro-organisms in degrading the environment contaminated depends on the type of micro-organisms, the type of contaminant and on the nature of the contaminated site. From the present study *Aspergillus niger* show efficient degrading capabilities by degrading organic contaminants as they use it for their growth and reproduction. In addition to the organic contaminant (carbon source) the micro-organisms require nitrogen and phosphorous as primary nutrient and traces of inorganic salts, through a series of complex enzymatically catalysed reactions, the toxic organic contaminant is converted to innocuous chemical compounds, obtain energy by catalysing energy producing chemical reactions and this energy is used in the production of new cells (Goudar & Subramanian, 1996) finally resulting in carbondioxide and water.

Hence biodegradation seems to be the most promising technique for industry treated brewery effluent as confirmed in the present investigation. Though the effluent is already treated by the industry, the results of physicochemical parameters of the above study indicate that it still has organic and inorganic pollutants above the permissible limits. Release of such industry treated effluents along with non-bio-degradable substances into the water bodies make the water unfit for human or agricultural or piscicultural use. After degradation using *Aspergillus* niger the treated effluent can be used for crop cultivation or irrigation purposes. The results of this study has shown that the biological treatment almost satisfied the irrigation water guideline.

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